## Remarks

The Office Action mailed October 1, 2002, has been received and reviewed. Claims 1-34. 36 and 37 having been canceled, and claims 35 and 38 having been amended, the pending claims are claims 35 and 38-43. Reconsideration and withdrawal of the rejections are respectfully requested.

The specification has been amended at pages 10-11 to format the line spacing of Tables 3-5 at 1.5 spaces per line. The specification has also been amended at page 26, line 9, to delete an unintentional recitation of a hyperlink. Finally, the specification has been amended at page 44, line 33 to correct a typographical error. The specification as amended correctly states that, excluding the N-terminal methionine, there are *four* methionines in each molecule. The amendment is supported, for example, by Figure 9 and the sequence listing (e.g., SEQ ID NO:1).

Claims 35 and 38 have been amended to further clarify the claims and to define abbreviations, which are also defined in the specification at, for example, page 12, line 11 to page 13, line 4.

## Objection to Disclosure

The Examiner objected to the disclosure for containing an embedded hyperlink. The disclosure has been amended by deleting the unintentional recitation of a hyperlink, and the objection is obviated.

The Examiner also objected to the line spacing on pages 10-11, Tables 3-5. The specification has been amended at pages 10-11 to format the line spacing of Tables 3-5 at 1.5 spaces per line, and the objection is obviated.

Applicants respectfully request that the Examiner withdraw the objection to the specification.

**Objection to Claims**

The Examiner objected to claims 35-38 for utilizing abbreviations, alleging that the use of abbreviations in claims is improper. Applicants respectfully traverse the objection. Claims 36 and 37 have been cancelled, rendering the objection moot with respect to those claims.

"[T]he terms and phrases used in the claims must find clear support or antecedent basis in the description so that the meaning of the terms in the claims may be ascertainable by reference to the description." 37 C.F.R. §1.75(d)(1). Thus, Applicants respectfully submit that the rules do not preclude the use of abbreviations in claims. Moreover, the M.P.E.P. even anticipates the use of abbreviations in claims ("Periods may not be used elsewhere in the claims except for abbreviations." M.P.E.P. §608.01(m).

The Examiner objected to the use of the abbreviations PEG, DMSO, *S. aureus*, and NAD. Applicants respectfully submit that the meanings of these abbreviations are clear to one of skill in the art. Furthermore, the abbreviations are all defined in the specification at, for example, page 12, line 11 to page 13, line 4.

However, in the interest of expediting the prosecution of the present application, claims 35 and 38 have been amended to incorporate the unabbreviated form at the first occurrence of each abbreviation in each claim. In view of the remarks offered herein, Applicants respectfully request that the objection to the claims be reconsidered and withdrawn.

**Rejections under 35 U.S.C. §112, Second Paragraph**

The Examiner rejected claims 35-37 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner stated that the phrase "molecular complex" is vague and indefinite. Applicants respectfully traverse the rejection. Claims 36 and 37 have been canceled, rendering the rejection moot with respect to those claims.

Applicants respectfully submit that the phrase "molecular complex" is clear and definite when read in view of the specification at, for example, page 25, lines 24-25. However,

in the interest of expediting the prosecution of the present application, claim 35 has been amended to delete the recitation of "molecular complex, " and the rejection is obviated.

Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. §112, second paragraph.

**Rejections under 35 U.S.C. §112, First Paragraph**

*ENABLEMENT*

The Examiner rejected claims 35-43 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner alleged that Applicants have failed to indicate suitable conditions for the crystallization of *Staphylococcus aureus* nicotinamide adenine dinucleotide. The Examiner states that "[p]roper disclosure for the conditions for crystallization (i.e., pH, concentration of protein, reagents, etc) is required for enablement" (page 4, lines 15-17 of the Office Action mailed October 1, 2002). Applicants respectfully traverse the rejection. Claims 36 and 37 have been canceled, rendering the rejection moot with respect to those claims.

Claim 35, as amended, recites:

35. A method for crystallizing *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase comprising:
providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and
crystallizing *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight polyethylene glycol (PEG) and about 0% by weight to about 20% by weight dimethyl sulfoxide (DMSO).

This embodiment of the method of the invention is explicitly set forth in the specification at page 9, lines 8-13. Moreover, although not required, the specification includes a working example (Example I) that directly supports the claimed method by describing

crystallization of *S. aureus* NAD synthetase using 15% PEG 1500 (page 41, lines 10-11), 16-22% PEG (page 41, lines 20-21) and 18-22% PEG (page 42, lines 6-7). Crystallization conditions were described, and certainly they are "suitable" as they led to the formation of crystals. The fact that, in detailing the crystallization process, the Applicants also set forth factors discovered during optimization that made crystallization more difficult does not mean that "suitable conditions" were not described. Furthermore, although crystallization conditions using PEG without salt or additional PH control were found to be preferred, it should also be noted that crystallization "hits" were observed under conditions that included pH control and ammonium sulfate, in addition to PEG (specification at page 41, lines 7-9).

Claims 38-43 are directed to a crystal of *S. aureus* NAD synthetase. Applicants submit that, in enabling claim 35, drawn to a method for making a crystal of *S. aureus* NAD synthetase, claims directed to the crystal (claims 38-43) are likewise enabled.

Applicants therefore respectfully submit that the claimed subject matter is described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

*WRITTEN DESCRIPTION*

The Examiner also rejected claim 43 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time of the application was filed, had possession of the claimed invention. Specifically, the Examiner alleged that SEQ ID NO:1 includes numerous methionines, that the specification fails to indicate which methionine(s) (by position) are replaced (individually and/or in combination), and that one of skill in the art would at least expect such replacement to result in structurally different coordinates. Applicants respectfully traverse the rejection.

Claim 43 recites a crystal of *Staphylococcus aureus* nicotinamide adenine dinucleotide (*S. aureus* NAD) synthetase having amino acid sequence SEQ ID NO:1, with the proviso that at least one methionine is replaced with selenomethionine. Applicants note that SEQ ID NO:1 includes an N-terminal methionine (residue 1) and four non-terminal methionines (e.g., residues 13, 26, 64, and 143). Although not required, Applicants have provided a working example of a crystal of *S. aureus* NAD synthetase having amino acid sequence SEQ ID NO:1, with the proviso that at least one methionine is replaced with selenomethionine (e.g., specification at, for example, page 40, lines 3-19; page 42, lines 23-25; page 43, line 24 to page 45, line 8; and page 47, lines 1-5). The specification (as amended) characterizes the selenomethionine enriched material by stating, for example, that "[t]here are four methionines in each molecule of NadE excluding the N-terminal methionine. Therefore, the eight [selenium] heavy atom positions would be consistent with the presence of two molecules in the asymmetric unit" (page 44, line 33 to page 45, line 2). Thus, the specification characterizes the selenomethionine enrichment at any or all of the four non-terminal methionines. Based on the arguments presented herein, Applicants respectfully submit that the specification reasonably conveys to one skilled in the relevant art that the inventor(s), at the time of the application was filed, had possession of the claimed invention.

Moreover, Applicants respectfully disagree with the Examiner's assertion that one of skill in the art would at least expect such replacement to result in structurally different coordinates. First, replacement of methionine by selenomethionine is well known in the art of protein crystallography as a method of incorporating heavy atoms into a molecule to aid in the solution of the x-ray crystal structure for the native molecule (e.g., without selenomethionine). The technique is useful *specifically* because the incorporation of the heavy atoms does not substantially disrupt the crystal structure. *See, for example*, Hendrickson et al., "Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure," *EMBO J.*, 9:1665-1672 (1990) (listed on a 1449 form included in the Information Disclosure Statement submitted herewith). Furthermore, the intent of the Examiner's statement that one of skill in the art would

at least expect such replacement to result in structurally different coordinates is unclear, as claim 43 does not recite specific structure coordinates. Clarification is respectfully requested in the next Official Communication.

Based on the remarks presented herein above, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. §112, first paragraph.

**Rejections under 35 U.S.C. §102**

The Examiner rejected claims 35-40 under 35 U.S.C. §102(b) as allegedly being anticipated by Rizzi et al. ("Crystallization of $NAD^+$ Synthetase from *Bacillus subtilis*," *Proteins: Structure, Function, and Genetics.* 1996; *26*:236-238). Applicants respectfully traverse the rejection. Claims 36 and 37 have been canceled, rendering the rejection moot with respect to these claims.

"[F]or anticipation under 35 U.S.C. 102, the reference must teach *every aspect* of the claimed invention either explicitly or impliedly." M.P.E.P. §706.02 (emphasis added). Applicants respectfully submit that Rizzi et al. fail to teach every aspect of the presently claimed invention (e.g., claims 35-40).

Independent claims 35-38 each recite *Staphylococcus aureus* nicotinamide adenine dinucleotide synthetase. In contrast, Rizzi et al. discloses *Bacillus subtilis* nicotinamide adenine dinucleotide synthetase. The present specification discloses that *S. aureus* NAD synthetase (SEQ ID NO:1) has a different amino acid sequence than *B. subtilis* NAD synthetase (SEQ ID NO:2) as illustrated, for example, in Figure 9 and the sequence listings. Moreover, the present specification discloses that crystals of *S. aureus* NAD synthetase differ from crystals of *B. subtilis* NAD synthetase as illustrated, for example, in Figure 7, Figure 8 (e.g., r.m.s. deviation between the Cα carbons is 1.19Å), and Figure 10.

Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. §102(b).

**Information Disclosure Statement**

Applicants thanks the Examiner for considering and initialing the documents cited on the 1449 forms submitted by Applicants with an Information Disclosure Statement mailed on December 21, 2001. However, on one of the 1449 forms (page 3 of 5, Exhibit A), the Examiner only initialed the first listed document. For the Examiner's convenience, Applicants are submitting herewith a clean copy of the 1449 form (page 3 of 5, Exhibit B). Consideration of each of the documents listed on the attached 1449 form (Exhibit B) is respectfully requested. Pursuant to the provisions of M.P.E.P. §609, Applicants further request that a copy of the 1449 form, marked as being considered and initialed by the Examiner, be returned with the next Official Communication.

## Summary

It is respectfully submitted that all the pending claims are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for

**Benson et al.**
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CERTIFICATE UNDER 37 CFR §1.8:
The undersigned hereby certifies that this paper is being transmitted by facsimile in accordance with 37 CFR §1.6(d) to the Patent and Trademark Office, addressed to Assistant Commissioner for Patents, Washington, D.C. 20231, on this 30th day of JANUARY, 2003, at 12:42 pm (Central Time).

By: _____
Name: SAM HER

**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE**
**Serial No.: 09/772,598**
**D cket No.: 6315.N**

Amendments to the following are indicated by double underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been indicated by the use of bold font.

**In the Specification**

Please delete the paragraph beginning at page 10, immediately following line 4 (Table 3).

Please insert the following new paragraph at page 10, immediately following line 4 (Table 3).

| | | | | |
|---|---|---|---|---|
| Molecule #1 | LEU 47 | LEU 83 | GLU 165 | LEU 214 |
| | GLY 48 | PHE 132 | PHE 170 | HIS 260 |
| | ILE 49 | ASN 136 | TYR 171 | LYS 261 |
| | SER 50 | ARG 140 | THR 172 | TYR 266 |
| | SER 55 | ARG 142 | LYS 173 | |
| | VAL 81 | THR 160 | ASP 176 | |
| Molecule #2 | TYR 1035 | PHE 1147 | SER 1151 | ASP 1180 |

Please delete the paragraph beginning at page 10, immediately following line 7 (Table 4).

Please insert the following new paragraph at page 10, immediately following line 7 (Table 4).

| | | | | |
|---|---|---|---|---|
| Molecule #1 | LEU 47 | GLN 88 | GLU 165 | GLU 215 |
| | GLY 48 | VAL 94 | GLY 169 | LEU 221 |
| | ILE 49 | ILE 111 | PHE 170 | ASP 223 |
| | SER 50 | PHE 132 | TYR 171 | ALA 226 |

| | | | | |
|---|---|---|---|---|
| | GLY 51 | GLY 135 | THR 172 | TYR 231 |
| | GLY 52 | ASN 136 | LYS 173 | ILE 256 |
| | GLN 53 | ALA 139 | TYR 174 | ARG 257 |
| | ASP 54 | ARG 140 | GLY 175 | ASN 258 |
| | SER 55 | ARG 142 | ASP 176 | ALA 259 |
| | THR 56 | GLN 146 | GLY 177 | HIS 260 |
| | VAL 81 | VAL 158 | LYS 189 | LYS 261 |
| | LYS 82 | GLY 159 | THR 211 | ALA 265 |
| | LEU 83 | THR 160 | ALA 212 | TYR 266 |
| | PRO 84 | ASP 161 | ASP 213 | TRP 271 |
| | TYR 85 | HIS 162 | LEU 214 | |
| Molecule #2 | TYR 1035 | GLN 1146 | SER 1151 | ALA 1179 |
| | HIS 1039 | PHE 1147 | GLY 1155 | ASP 1180 |
| | PHE 1041 | SER 1148 | ILE 1156 | ILE 1181 |
| | ILE 1042 | ALA 1150 | VAL 1157 | |

Please delete the paragraph beginning at page 11, immediately following line 2 (Table 5).

Please insert the following new paragraph at page 11, immediately following line 2 (Table 5).

| | | | | |
|---|---|---|---|---|
| Molecule #1 | VAL 46 | VAL 94 | THR 160 | ASP 217 |
| | LEU 47 | GLU 95 | ASP 161 | LYS 218 |
| | GLY 48 | ALA 97 | HIS 162 | LEU 221 |
| | ILE 49 | LEU 98 | ALA 164 | PRO 222 |

| | | | | |
|---|---|---|---|---|
| | SER 50 | ILE 101 | GLU 165 | ASP 223 |
| | GLY 51 | VAL 107 | ASN 166 | GLU 224 |
| | GLY 52 | THR 108 | THR 168 | ASP 225 |
| | GLN 53 | VAL 109 | GLY 169 | ALA 226 |
| | ASP 54 | ASN 110 | PHE 170 | LEU 227 |
| | SER 55 | ILE 111 | TYR 171 | TYR 231 |
| | THR 56 | THR 130 | THR 172 | HIS 254 |
| | LEU 57 | ASP 131 | LYS 173 | TYR 255 |
| | VAL 58 | PHE 132 | TYR 174 | ILE 256 |
| | GLY 59 | GLN 133 | GLY 175 | ARG 257 |
| | PHE 78 | LYS 134 | ASP 176 | ASN 258 |
| | ILE 79 | GLY 135 | GLY 177 | ALA 259 |
| | ALA 80 | ASN 136 | ALA 178 | HIS 260 |
| | VAL 81 | GLU 137 | ALA 179 | LYS 261 |
| | LYS 82 | LYS 138 | ILE 184 | ARG 262 |
| | LEU 83 | ALA 139 | LYS 189 | GLU 263 |
| | PRO 84 | ARG 140 | LYS 208 | LEU 264 |
| | TYR 85 | GLU 141 | THR 209 | ALA 265 |
| | GLY 86 | ARG 142 | PRO 210 | TYR 266 |
| | VAL 87 | MET 143 | THR 211 | THR 267 |
| | GLN 88 | VAL 145 | ALA 212 | THR 270 |
| | LYS 89 | GLN 146 | ASP 213 | TRP 271 |
| | ASP 90 | VAL 157 | LEU 214 | PRO 272 |
| | ALA 91 | VAL 158 | GLU 215 | |

| | GLU 93 | GLY 159 | ASP 216 | |
|---|---|---|---|---|
| Molecule #2 | PHE 1031 | LEU 1045 | ALA 1150 | GLY 1159 |
| | TYR 1035 | VAL 1046 | SER 1151 | ALA 1178 |
| | VAL 1036 | MET 1143 | ASN 1152 | ALA 1179 |
| | SER 1038 | LYS 1144 | ARG 1153 | ASP 1180 |
| | HIS 1039 | VAL 1145 | GLN 1154 | ILE 1181 |
| | SER 1040 | GLN 1146 | GLY 1155 | ALA 1182 |
| | PHE 1041 | PHE 1147 | ILE 1156 | |
| | ILE 1042 | SER 1148 | VAL 1157 | |
| | SER 1044 | ILE 1149 | VAL 1158 | |

The paragraph beginning at page 25, line 22, has been amended as follows:

The structure coordinates set forth in Table 1 can be used to aid in obtaining structural information about another crystallized molecule or molecular complex. A "molecular complex" means a protein in covalent or non-covalent association with a chemical entity or compound. The method of the invention allows determination of at least a portion of the three-dimensional structure of molecules or molecular complexes which contain one or more structural features that are similar to structural features of *S. aureus* NadE. These molecules are referred to herein as "structurally homologous" to *S. aureus* NadE. Similar structural features can include, for example, regions of amino acid identity, conserved active site or binding site motifs, and similarly arranged secondary structural elements (e.g., α helices and β sheets). Optionally, structural homology is determined by aligning the residues of the two amino acid sequences to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in

their proper order. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.9, of the BLAST 2 search algorithm, as described by Tatusova et al., FEMS Microbiol Lett 174, 247-50 (1999), and available **from the world wide web** at **[http://www.]**ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as "identity." Preferably, a structurally homologous molecule is a protein that has an amino acid sequence sharing at least 65% identity with the amino acid sequence of *S. aureus* NadE (SEQ ID NO: 1). More preferably, a protein that is structurally homologous to *S. aureus* NadE includes at least one contiguous stretch of at least 50 amino acids that shares at least 80% amino acid sequence identity with the analogous portion of *S. aureus* NadE. Methods for generating structural information about the structurally homologous molecule or molecular complex are well-known and include, for example, molecular replacement techniques.

The paragraph beginning at page 44, line 24, has been amended as follows:

The resolution and quality of the anomalous and dispersive signals were poor making data interpretation difficult. A second data set collected on a selenomethionine incorporated NadE crystal grown in the presence of $N^aAD$ was also very mosaic suggesting a fundamental problem with the selenomethionine crystals and/or the cryogenic conditions used to preserve the crystals. However, the data was of sufficient quality at 2.7Å to begin to identify heavy atom positions. Both anomalous and dispersive difference Patterson maps revealed the presence of at least four strong heavy atom peaks (Figures 2-3). A total of eight sites were identified using automated Patterson interpretation methods in SHELX (Sheldrick et al., Acta Cryst., B51:423-31 (1995)). There are **[five]four** methionines in each molecule of NadE excluding the N-terminal methionine. Therefore, the eight heavy atom positions would be consistent with the presence of two molecules in the asymmetric unit. Phasing with these eight sites led to electron density

maps that were difficult to interpret suggesting that the positions of the heavy atom sites might be incorrect except for the fact that these sites were completely consistent with the Patterson maps. Methionine positions from the initial molecular replacement solutions described above using the NadE dimer from *Bacillus subtilis* were also consistent with the peaks in the Patterson maps making the difficulty in refinement and the low quality electron maps even more puzzling.

**In the Claims**

For convenience, all pending claims are shown below.

35. **(Amended)** A method for crystallizing **[an]** ***Staphylococcus aureus*** **nicotinamide adenine dinucleotide (***S. aureus* NAD) synthetase **[molecule or molecular complex]** comprising:
providing purified *S. aureus* NAD synthetase at a concentration of about 1 mg/ml to about 50 mg/ml; and

crystallizing *S. aureus* NAD synthetase from a solution comprising about 5% by weight to about 50% by weight **polyethylene glycol (**PEG) and about 0% by weight to about 20% by weight **dimethyl sulfoxide (**DMSO).

38. **(Amended)** A crystal of ***Staphylococcus aureus*** **nicotinamide adenine dinucleotide (***S. aureus* NAD) synthetase.

39. The crystal of claim 38 having the trigonal space group symmetry $P2_1$.

40. The crystal of claim 38 comprising a unit cell having dimensions of a, b, and c; wherein a is about 40Å to about 60Å, b is about 90Å to about 120Å, and c is about 80Å to about 110Å; and wherein $\alpha = \gamma = 90°$ and $\beta$ is about 80° to about 120°.

41. The crystal of claim 38 comprising atoms arranged in a spatial relationship represented by the structure coordinates listed in Table 1.

42. The crystal of claim 38 having amino acid sequence SEQ ID NO:1.

43. The crystal of claim 38 having amino acid sequence SEQ ID NO:1, with the proviso that at least one methionine is replaced with selenomethionine.